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Effectiveness of Vitamin C Supplementation on Bone Mineral Density and Peripheral Arterial Quality

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ABSTRACT

Background: Vitamin C is an essential nutrient vital for optimal human health. Previous findings have indicated the potential benefits of vitamin C on bone mineral density (BMD) and peripheral arterial quality through various mechanical pathways. However, the effectiveness of vitamin C supplementation on BMD and peripheral arterial quality remains controversial. **Methods:** A total of 60 healthy participants were recruited and randomly allocated into control (n=30) and intervention (n=30) groups. Background data such as body composition were collected from all participants using BODPOD. Additionally, BMD measurements of wrist and ankle were obtained using an Osteo checker device, and peripheral arterial quality was assessed using the Ankle Brachial Index (ABI) device at baseline. The intervention group received daily vitamin C supplementation of 100 mg per 1 tablet for 12 weeks, whereas the control group maintained their routine dietary habits. All parameters were reassessed at week 12 (endpoint). **Results:** At endpoint, ankle BMD values in the intervention group were significantly higher than in the control group (P<0.05). Moreover, the intervention group demonstrated significantly higher peripheral arterial quality in both right and left brachial ABI and right and significantly lower left ankle Arterial Stiffness Index (ASI) compared to the control group at endpoint (P<0.05). Dietary habits, including energy and calcium intake, showed no significant differences between groups. **Conclusions:** Daily vitamin C supplementation of 100 mg appeared to be potentially effective for improving BMD and ABI values among participants in this study. However, further study is needed with larger participant groups and longer duration to confirm these findings.

Introduction

Vitamin C (Ascorbic acid) is vital for numerous physiological functions, and its deficiency can lead to scurvy, immune dysfunction, and cartilage abnormalities (Maxfield *et al.*, 2025). The recommended intake ranges from 40 to 120 mg/day, depending on age and sex (Langlois *et al.*, 2019). However, previous studies have demonstrated that vitamin C supplementation beyond the DRI, particularly at 100 mg/day, may be associated with reduced risks of all-cause mortality, cardiovascular disease, and several cancers, including esophageal, gastric, cervical,

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and lung (Ashor *et al.*, 2019, Xu *et al.*, 2022). In Thailand, inadequate vitamin C intake is common due to low fruit and vegetable consumption (Boonpangrak *et al.*, 2018). Enhancing intake through diet or supplementation may help reduce deficiency-related health risks. Several studies have established that vitamin C may contribute to increased bone mineral density (BMD) through its antioxidant properties (Aghajanian *et al.*, 2015, Malmir *et al.*, 2018). This mechanism operates via the promotion of immunosuppressive cytokine IL-10 secretion by dendritic cells (DCs), subsequently inhibiting proinflammatory cytokine production. Additionally, vitamin C has been shown to inhibit NF-κB dependent IL-6 production, a cytokine known to mediate inflammatory responses and stimulate osteoclast, thereby elevating bone turnover (Boyce *et al.*, 2023, Sasidharan Nair and Huehn, 2024, Sellin *et al.*, 2023). The study by Mangano et al. has demonstrated a positive correlation between sufficient serum vitamin C levels and higher BMD scores (Mangano *et al.*, 2021). Furthermore, the study has shown that inadequate vitamin C intake is associated with lower BMD and an elevated risk of osteoporosis development (Ratajczak *et al.*, 2020). However, further studies are needed to confirm these findings regarding the relationship between vitamin C and BMD levels.

The Ankle Brachial Index (ABI) is a non-invasive and accurate method established by previous studies for determining peripheral arterial quality and screening for arterial stiffness (Casey *et al.*, 2019, Rac-Albu *et al.*, 2014). The optimal ABI value ranges from 0.9 to 1.40, whereas an ABI value <0.9 indicates risk of PAD. Additionally, this method can determine the Arterial Stiffness Index (ASI), for which the optimal value is less than 70; therefore, an ASI score above 70 indicates risk of arterial stiffness development (Cáceres-Farfán *et al.*, 2021). Vitamin C is a crucial cofactor in collagen biosynthesis (DePhillipo *et al.*, 2018) which is essential for maintaining blood vessel structural integrity (Zhao *et al.*, 2022). Vitamin C deficiency can lead to compromised vascular integrity and reduced elasticity, as demonstrated by in vivo studies (Skovsted *et al.*, 2022). However, there is limited empirical evidence regarding the effects of vitamin C on ABI improvement. Similarly, data on the relationship between vitamin C supplementation and BMD remains sparse. Furthermore, the optimal dose of vitamin C required to influence ABI and BMD scores has not been clearly established. Therefore, this study aimed to investigate the effectiveness of vitamin C supplementation on BMD and ABI scores among healthy Thai adults, using a dosage that has been shown to be effective on health parameters in previous studies.

Material and Methods

This study was conducted through quota sampling from June 2024 - April 2025 with a total of 60 healthy Thai adults who were residing in Sansook Municipality, Muang District, Chonburi province, Thailand. The study instruments were as follows:

Basic information questionnaire

A closed-ended questionnaire was designed and developed to collect background data from participants, including gender, age, body mass index (BMI), education level, weekly exercise duration, length of daily sun exposure, and etc. The participants completed this questionnaire independently.

Three-day food record questionnaire

A closed-ended questionnaire was developed for participants to record their food intake for three days after joining the study and receiving education about food portion estimation. Participants were asked to record their food consumption for two weekdays and one weekend day during the final week of the study. This data was used for calculating energy intake and nutrient consumption to obtain information about energy distribution, daily vitamin C, and daily calcium (Ca) intake. The food energy intake data, vitamin C and Ca contents were calculated using the nutritional software package, INMUCAL-Nutrient version 4.0, developed by the Institute of Nutrition, Mahidol University, Thailand.

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Vitamin C dietary supplement

The vitamin C supplement product contained 100 mg (Hicee®, Thailand Food and Drug Administration registration number 2A 33/48, which are equivalent to the dosage used in the study by Brzezińska *et al*. that showed significant health benefits. Participants received vitamin C supplements to consume one tablet daily after lunch. Consumption adherence was monitored through the Line application to ensure compliance (Brzezińska *et al.*, 2020).

Peripheral arterial function measurement device

This peripheral arterial function evaluation device (HBP-8000, OMRON, Kyoto, Japan) was used to measure peripheral arterial function and underwent regular calibration and maintenance by expert technicians to ensure measurement accuracy. The device measured ABI values of peripheral arteries in different segments, including left and right arms and legs, with results printed from the device.

Bone density measurement device

This equipment was used to measure bone density in wrists and ankles using dual-energy X-ray absorptiometry (DEXA) technology. The Osteo Checker (Ampall Co. Ltd., Seoul, South Korea) was calibrated before each use to ensure data reliability.

Body composition analysis devices

The InBody270 (InBody Co., Seoul, South Korea) and BODPOD (COSMED Co., Rome, Italy) devices were regularly calibrated and utilized for measuring participants' body composition. Body composition parameters, including percent body fat, muscle mass, and thoracic gas volume, were determined in all participants at both baseline and endpoint to monitor changes in body composition throughout the study period.

Study participants

Study participants were conveniently sampled from the general population residing in Sansook Municipality, Muang District, Chonburi Province based on a power analysis conducted using G*Power with a power of 0.80 (Brzezińska *et al.*, 2020). After enrollment, 54 participants were set as the primary number of calculated subjects, which included the dropout rate for 20%. Therefore, the participants in this study were 60. After recruitment, they were then randomly allocated into either the control group (n=30) or the intervention group (n=30) using quota sampling. The inclusion criteria comprised individuals of Thai ethnicity and nationality who were in good health without chronic diseases, were legally adults aged between 18-60, and possessed Thai language literacy skills. The exclusion criteria encompassed individuals with severe communicable diseases, oral health problems affecting mastication and swallowing, current consumption of dietary supplements or herbal products, pregnant or lactating women, persons with cognitive impairments affecting communication, participants who withdrew from the study, and those who provided incomplete questionnaire responses or data.

Study procedures

During the study procedure, participants were invited to a designated room at the Nutrition and Dietetics Laboratory, Faculty of Allied Health Sciences, Burapha University, Chonburi Province. The study protocol was explained in detail, and each participant signed an informed a consent form. Following group allocation, baseline measurements were collected during the week prior to the intervention's commencement. These measurements were conducted using equipment from the Nutrition and Dietetics Division, Faculty of Allied Health Sciences, Burapha University, which included body weight, height, peripheral arterial function using the ABI device, and body composition parameters (percent body fat, muscle mass, and body fluid percentage) using BODPOD and InBody analyzers. Wrist and ankle bone mass were measured using the Osteo checker device. Participants then completed a basic information questionnaire and received education about food exchange lists using food models as teaching aids. This education prepared them for completing the three-day food record questionnaire, which was distributed to each

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participant. They were instructed to record their food intake for two weekdays and one weekend day during the final week of the study.

In the subsequent phase, the intervention group received vitamin C supplements (100 mg per tablet) and were instructed to consume one tablet daily after lunch for 12 consecutive weeks. To ensure compliance, participants in both groups were monitored through line application, with the intervention group required to send photographs of their supplement consumption. The control group was instructed to maintain their food records but did not receive vitamin C supplementation. They were scheduled for follow-up measurements of various parameters, including ABI and bone mass, at week 12 (Brzezińska *et al.*, 2020).

In the final week (week 12), participants from both groups were requested to return for submission of their three-day food records, which were used to calculate energy intake, energy distribution, vitamin C, and Ca consumption for each participant. Final measurements were conducted, including peripheral arterial function using the ABI device, wrist and ankle bone mass measurements, and body composition analysis using BODPOD and InBody devices. These final week data were collected for subsequent statistical analysis.

Ethical considerations

All questionnaires were reviewed by three nutrition experts to ensure validity and confidentiality. The study protocol received ethical approval from the Institutional Human Research Ethics Committee of Burapha University (Approval No. IRB1-118/2567). This study is registered with the Thai Clinical Trials Registry (TCTR20250506006). Moreover, the protocol of the study was conducted at the Nutrition and Dietetics Laboratory, Faculty of Allied Health Sciences, Burapha University, Chonburi Province.

Data analyses

Demographic data including gender, income levels, and educational background, were presented as percentages, and differences in these proportions between groups were analyzed using Fisher's exact test. BMI, weekly exercise duration, daily sun exposure, energy intake, energy distribution, vitamin C and Ca intake, peripheral arterial function indicated by ABI and ASI values, t-scores, BMD values of wrist and ankle bones, and various body composition parameters were expressed as mean ± standard deviation (mean ± SD). Between-group comparisons of these variables were conducted using independent paired t-tests, while within-group comparisons of changes between baseline and endpoint measurements were analyzed using simple paired t-tests. Statistical analyses were performed using Predictive Analytics Software Statistics (SPSS Inc, Chicago, IL), version 25.0, with statistical significance set at P-value<0.05.

Results

Participants' background and characteristics

Figure 1 shows the flow chart of the study. As can be seen in this figure, all the participants who entered the study at the beginning did not drop out until the end of the study.

For participants' background, there were no significant differences in participant background characteristics between groups. The majority of participants were female in both the control (64%) and intervention (77%) groups. The mean ages of the control and intervention groups were 22.26 and 23.13 years, respectively. Most participants were part-time employees (80% for control and 73% for intervention groups) and held Bachelor's degrees (90% for control and 77% for intervention groups). Additionally, the mean weekly exercise duration and daily sun exposure were 78.66 and 35.56 minutes in the control group, and 97.16 and 41.66 minutes in the intervention group, respectively (**Table 1**).

Effects of vitamin C supplementation on BMD values

The effect of vitamin C supplementation on BMD was evaluated through wrist and ankle measurements. At the endpoint, participants in the intervention group showed no significant differences compared to the control group across most parameters. However, ankle BMD values were significantly higher in the intervention group (0.46

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g/cm^2) compared to the control group (0.43 g/cm^2; *P*<0.05). Furthermore, within the intervention group, ankle BMD values significantly increased from baseline (0.41 g/cm^2) to endpoint (0.46 g/cm^2; *P*<0.05, **Table 2**).

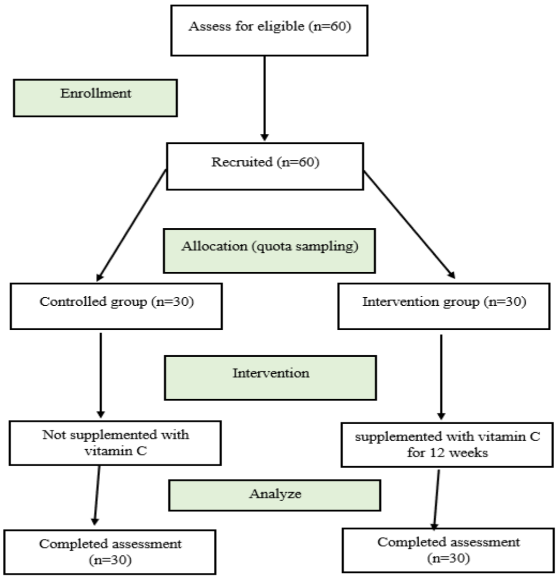

Figure 1. Consort flowchart of the study.

Effects of vitamin C supplementation on peripheral arterial quality

The ABI and ASI values were measured to assess peripheral arterial quality. At the endpoint, participants in the intervention group showed significantly higher right and left ABI scores (1.01 and 0.98, respectively) compared to the control group (0.93 and 0.90, respectively; *P*<0.05). Furthermore, the right and left ankle ASI values in the intervention group (86.70 and 77.50, respectively) were significantly lower than those in the control group (97.06 and 91.26, respectively; *P*<0.05) at the endpoint. Within-group analysis revealed that participants in the intervention group demonstrated significant improvements in peripheral arterial quality scores, with increased right ABI (baseline=0.91, endpoint=1.01; *P*<0.05) and decreased right brachial ASI (baseline=57.36, endpoint=53.73; *P*<0.05), right ankle ASI (baseline=100.76, endpoint=86.70; *P*<0.05), and left ankle ASI (baseline=90.23, endpoint=77.50; *P*<0.05) at the endpoint (**Table 3**).

Changes in participants' body composition were analyzed using body composition analyzer devices. The results showed no significant changes in most body composition parameters from baseline to endpoint, and no significant differences between groups. However, mineral body mass was significantly higher in the intervention group (2.94 kg) compared to the control group (2.63 kg; *P*<0.05) at the endpoint. Additionally, the intervention group showed a significant increase in mineral body mass from baseline (2.61 kg) to endpoint (2.94 kg; *P*<0.05, **Table 4**).

Table 1. Participants' background and characteristics of both groups.

Parameters	**Control group (n=30)**	**Intervention group (n=30)**	**P-value**
Sex			
Male	11 (36) [c]	7 (23)	0.39[a]
Female	19 (64)	23 (77)	
Age (y)	22.26±1.99[d]	23.13±2.33	0.12[b]
Occupation			
Part-time employee	24 (80)	21 (73)	0.76[a]
Businessperson	6 (20)	9 (27)	
Education			
Bachelor's degree	27 (90)	23 (77)	0.29[a]
Graduate degree	3 (10)	7 (23)	
Length of weekly exercise (minute)	78.66±77.09	97.16±70.75	0.33[b]
Length of daily sun exposure (minute)	35.56±17.94	41.66±24.65	0.27[b]

[a]: *Fisher's exact test;* [b]: *Independent t-test;* [c]: *n(%);* [d]: *Mean±SD.*

Discussion

Vitamin C supplementation has been shown to have multiple health benefits as reported in previous studies, including anti-inflammatory effects, immune system enhancement, reduction of allergic symptoms, and maintenance of muscle mass (Chambial *et al.*, 2013). Prior study has indicated that vitamin C deficiency is associated with increased osteoclastogenesis through elevated RANK expression (Park *et al.*, 2012). The findings of the present study suggest that vitamin C supplementation tends to enhance participants' BMD, specifically evidenced by increased BMD values in the ankle region. Potential confounding

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factors were controlled for, as there were no significant differences between groups in variables known to affect BMD, such as weekly exercise duration, daily sun exposure, and calcium intake (Kongpharm *et al.*, 2024, Lee *et al.*, 2021, Min *et al.*, 2021). The observed results may be attributed to vitamin C's role in stimulating osteoblast activity and its involvement in inhibiting inflammatory cytokines that stimulate osteoclast activity and bone turnover through the Wnt/β-Catenin/ATF4 signaling pathways (Choi *et al.*, 2019). Notably, the ankle was the only measured skeletal site that showed significant improvement in the intervention group. This localized effect may be due to the ankle's regular weight-bearing function, which is known to stimulate bone formation (Zhang *et al.*, 2017). These findings from the present study align with previous studies demonstrating the positive effects of weight-bearing and resistance exercise on bone formation stimulation (Benedetti *et al.*, 2018, Hong and Kim, 2018).

Table 2. BMD values of participants at baseline to endpoint in both groups.

Parameters	Control group (30)	Intervention group (30)	P-value[b]
T-score of wrist			
Baseline	-1.24±	-1.09±1.06	0.56
Endpoint	0.90[a]	-1.03±1.02	0.71
P-value[c]	-1.12±0.94	0.82	
	0.61		
BMD of wrist			
(g/cm^2)	0.44±0.04	0.46±0.05	0.30
Baseline	0.46±0.05	0.49±0.08	0.10
Endpoint	0.09	0.08	
P-value			
T-score of			
ankle	-1.00±1.13	-0.87±1.17	0.75
Baseline	-0.94±1.30	-0.83±1.25	<0.05*
Endpoint	0.84	0.89	
P-value			
BMD of ankle			
(g/cm^2)	0.42±0.02	0.41±0.02	0.11
Baseline	0.43±0.03	0.46±0.03	<0.05*
Endpoint	0.90	<0.05*	
P-value			

[a]: Mean± SD; [b]: Independent t-test; [c]: Paired t-test; ***BMD****: Bone mineral density.*

Table 3. Peripheral arteries quality scores of participants at baseline to endpoint in both groups.

Parameters	Control group (30)	Intervention group (30)	P-value[b]
Right ABI			
Baseline	0.92±0.17	0.91±0.13	0.74
Endpoint	0.93±0.13	1.01±0.14	<0.05*
P-value[c]	0.79	<0.05*	
Left ABI			
Baseline	0.93±0.13	0.91±0.13	0.58
Endpoint	0.90±0.14	0.98±0.14	<0.05*
P-value	0.39	0.05	
Right			
brachial ASI	56.86±7.52	57.36±7.91	0.80
Baseline	55.96±7.53	53.73±8.30	0.28
Endpoint	0.64	0.09	
P-value			
Left brachial			
ASI	57.46±6.25	56.96±7.87	0.78
Baseline	56.90±5.52	56.20±7.78	0.69
Endpoint	0.71	0.70	
P-value			
Right ankle			
ASI	99.43±25.89	100.76±20.68	0.82
Baseline	97.06±22.44	86.70 ±12.93	<0.05*
Endpoint	0.70	<0.05*	
P-value			
Left ankle			
ASI	91.40±12.77	90.23±13.94	0.73
Baseline	91.26±13.12	77.50±13.02	<0.05*
Endpoint	0.96	<0.05*	
P-value			

[a]: Mean± SD; [b]: Independent t-test; [c]: Paired t-test; ***ABI****: Ankle Brachial Index;* ***ASI****: Arterial Stiffness Index.*

Three-day dietary records were collected to assess participants' dietary habits. The analysis revealed no significant differences between groups in the percentage distribution of energy from carbohydrates, proteins, and fats. Furthermore, there were no significant differences in daily Ca intake between groups. Vitamin C was the only nutrient that showed significant differences between groups. Participants in the intervention group, who received daily vitamin C supplementation, had a significantly higher daily vitamin C intake (180.63 mg) compared to the control group (75.76 mg; $P<0.05$, **Table 5**).

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Table 4. Body composition data of participants at baseline to endpoint in both groups.

Parameters	Control group (30)	Intervention group (30)	P-value[b]
Thoracic gas volume (l)			
Baseline	3.13±0.22	3.19±0.25)	0.36
Endpoint	3.08±0.21	3.14±0.18	0.22
P-value[c]	0.37	0.37	
Percentage of body fat			
Baseline	20.52±3.00	20.18±2.41	0.63
Endpoint	20.79±2.43	20.09±2.23	0.25
P-value	0.70	0.88	
Percentage of fat free mass			
Baseline	79.48±3.00	79.81±2.41	0.63
Endpoint	79.02±2.43	79.90±2.23	0.25
P-value	0.51	0.88	
Fat mass (kg)			
Baseline	16.64±2.35	17.56±1.20	0.06
Endpoint	16.84±2.49	17.75±1.74	0.10
P-value	0.75	0.62	
Body fluid (l)			
Baseline	30.84±3.29	30.64±3.18	0.81
Endpoint	30.65±3.44	31.08±2.93	0.60
P-value	0.82	0.57	
Muscle mass (kg)			
Baseline	19.98±3.33	19.70±2.95	0.73
Endpoint	19.82±3.25	19.42±2.93	0.62
P-value	0.85	0.71	
Weight from minerals (kg)			
Baseline	2.54±0.27	2.61±0.32	0.41
Endpoint	2.63±0.35	2.94±0.32	<0.05*
P-value	0.26	<0.05*	
Energy requirement (kcal)			
Baseline	1,802±200	1,817±194	0.80
Endpoint	1,837±205	1,843±169	0.90
P-value	0.50	0.58	

[a]: Mean± SD; [b]: Independent t-test; [c]: Paired t-test.

The ABI values showed a significant improvement bilaterally in the intervention group. This technique serves as an indicator of blood flow quality and potential vascular obstruction (Khan *et al.*, 2008). Although both groups maintained ABI values within normal ranges at baseline and endpoint measurements, the increased ABI values observed in the intervention group suggest that vitamin C supplementation may enhance vascular function.

This improvement was further supported by decreased ASI values in both right and left ankles among intervention group participants, indicating reduced peripheral arterial stiffness. These results can be attributed to vitamin C's vital role in collagen synthesis, the type of protein that support vascular endothelial cells functions (Lis *et al.*, 2022). Therefore, adequate vitamin C intake appears to be a significant factor in enhancing vascular function, particularly in terms of blood circulation and vessel elasticity. The results support previous findings indicating that vitamin C is essential for maintaining cardiovascular health, primarily through its role in supporting endothelial cell function, which is critical for vascular integrity. It contributes to improved blood flow, reduced vascular inflammation, and strengthened vascular walls. Furthermore, evidence from a meta-analysis of randomized controlled trials suggests that vitamin C supplementation may be effective in lowering blood pressure, particularly among individuals with hypertension (May and Harrison, 2013, Morelli *et al.*, 2020). However, maintaining a low dietary fat intake is also a key factor in keeping the blood lipid profile at an optimal level and preventing the risk of arterial stiffness.

Vitamin C supplementation did not significantly affect body composition parameters in this study, with the exception of mineral mass, which showed elevation in the intervention group. This finding may be attributed to vitamin C's potential to enhance the absorption of trace elements and minerals, such as iron and selenium, in the human body (Bae and Kim, 2020, Li *et al.*, 2020). Regarding dietary habits, nutrient intake was comparable between groups, with vitamin C being the only difference due to supplementation in the intervention group. Notably, vitamin C intake in the control group remained at borderline levels according to DRI recommendations of 75-90 mg per day for adults (Naidu, 2003). Similarly, calcium intake in both groups fell below the recommended daily allowance of 1,300 mg for

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adults (Cormick and Belizán, 2019). These findings support previous studies indicating that insufficient consumption of fruits, vegetables, and calcium-rich food sources represents a significant nutritional challenge (Roark and Niederhauser, 2013, Shlisky *et al.*, 2022, Singhato *et al.*, 2024, Singhato *et al.*, 2020). In contrast, some trace elements, such as selenium, were reported as adequate intake among people (Tiyawisutsri *et al.*, 2025). Several limitations of this study should be noted. Serum vitamin C levels were not measured, necessitating a future study to determine the effectiveness of vitamin C supplementation on serum concentrations and the studied parameters in larger cohorts over extended periods. Furthermore, future investigations should examine the combined effects of vitamin C supplementation with other factors, such as exercise, in comparison to vitamin C supplementation alone on these parameters. Moreover, determination of the blood lipid profiles due to its relationship to the arterial functions and investigating the BMD values of other parts of the body by a longer intervention term among a larger number of participants is suggested for future studies. Finally, this study did not have a placebo group, and other nutrients related to bone were not determined.

Table 5. Participants' dietary habits.

Nutrients consumed	Control group (n=30)	Intervention group (n=30)	P-value[a]
Percentage energy from carbohydrate	57.36±5.06	55.90±4.74	0.25
Percentage energy from protein	14.16±2.52	13.86±2.22	0.62
Percentage energy from fat	28.46±4.76	30.23±5.22	0.17
Daily vitamin C intake (mg)	75.76±18.80	180.63±16.76	<0.05
Daily Ca intake (mg)	570.66±165.15	627.06±166.68	0.19

[a]: *independent t-test.*

Conclusion

This study provides preliminary indications that daily supplementation with 100 mg of vitamin C for 12 weeks may be associated with modest improvements in BMD at the ankle and peripheral arterial function, as reflected by trends toward increased ABI values in healthy Thai adults. These potential effects appeared to occur independently of dietary energy and calcium intake, suggesting a possible role for vitamin C in supporting musculoskeletal and vascular health. However, due to the limited sample size and relatively short intervention period, these findings should be interpreted with caution. Further investigations involving larger, more diverse populations and longer follow-up durations are necessary to confirm and clarify the potential mechanisms underlying these associations.

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Authors' contributions

Kanchana N, Silarod P, Ruangpum B, Phengkrajang K, Chumjit O, Seesod P, Janhom P, Uaongnarat P, Benjaprom C, Petsiri W, Jueajaiamnart N, and Thanopajai N collected the data. Rueangsri N conceptualized the study, analyzed the data, and wrote the manuscript. Prapkree L conceptualized the study and proofread the manuscript. All authors read the manuscript and approved its publication.

Conflict of interest

The authors declared no conflict of interest.

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